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SUSCEPTIBILITY TO INFECTION WITH PASTEURELLA TULARENSIS AND THE IMMUNE RESPONSE OF MICE EXPOSED TO CONTINUOUS LOW DOSE RATE GAMMA RADIATION

by

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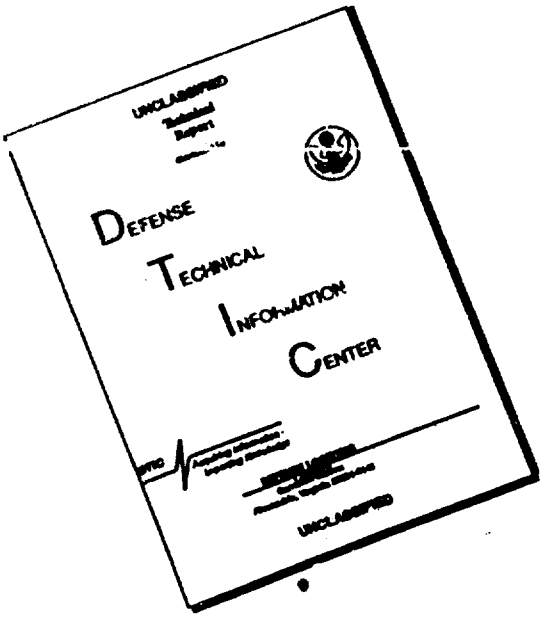

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ADMINISTRATIVE INFORMATION

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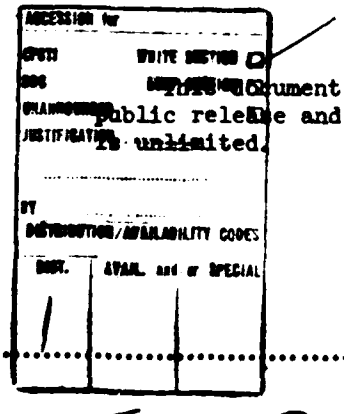

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ABSTRACT

Mice were exposed to continuous low dose rate (1.4 R/hr) γ radiation. Immediately after accumulating 1000 R, 2000 R, or 3000 R, they received a subcutaneous injection of the live vaccine strain (LVS) of Pasteurella tularensis. Although the avirulent strain caused no effect in normal mice, a fulminating infection occurred in irradiated mice. As the total radiation dose increased the LD_{50} of the organism decreased.

Animals surviving irradiation and immunization were subjected to an aerosol challenge of either the avirulent LVS or the virulent SCHU S-5 strain of P. tularensis. The LVS strain is virulent for mice when administered as an aerosol, but not when injected subcutaneously. Although an aerosol of avirulent organisms was lethal for normal mice, immune mice, both non-irradiated and irradiated, survived an aerosol infection with the avirulent organism. Irradiated, immunized mice challenged with an aerosol of the virulent SCHU S-5 strain showed a decrease in immunity, especially at the higher levels of radiation.

Bacterial counts of the lungs, liver, spleen, and lymph nodes of non-immunized mice exposed to a lethal aerosol dose of the avirulent P. tularensis (LVS) showed increasing numbers of bacteria after the 2nd post-infection day. This increase continued until the death of the animal on about the 10th day. Immune animals, however, were able to clear the lungs 5 days after infection with no involvement of the other tissues.

Although continuous low dose rate γ radiation does not produce the gross radiation pathology that one sees following acute radiation, exposure to a live avirulent Pasteurella tularensis vaccine produces an infection and an impaired immunity.

NON-TECHNICAL SUMMARY

The Problem

There is considerable military interest regarding the possibility that exposure to low dose rate gamma radiation, such as might be encountered in a radiation fallout field following the use of a nuclear weapon, may decrease the individual's resistance to live, avirulent bacterial vaccines. Live bacterial vaccines are of an additional interest since they provide a greater degree of immune protection than do killed vaccines. This also poses the question as to whether the individuals' ability to acquire immunity might be impaired after radiation exposure.

The Findings

Mice exposed to continuous low dose rate radiation became increasingly susceptible to an inoculation of the avirulent live vaccine strain (LVS) of organisms causing tularemia, Pasteurella tularensis. As the total radiation dose increased the per cent mortality of the infected mice increased. Non-irradiated mice were not affected, even after infection with high bacterial doses.

Animals surviving the radiation and the immunization were subjected to an aerosol challenge with either the avirulent live vaccine strain or the virulent SCHU S-5 strain of the tularemia organism. Although an aerosol of the avirulent organism was lethal for normal mice, there was no decrease in the immunity of irradiated, immunized mice compared with non-irradiated, immunized mice. Irradiated, immunized mice challenged with

an aerosol of the highly virulent SCHU S-5 strain however, showed a decrease in the immune response, especially at the higher radiation levels.

Studies on the growth of the organisms in the lung, liver, spleen, and lymph nodes of non-immunized mice infected with the avirulent aerosol, showed increasing numbers of bacteria. Immune animals, whether irradiated or not, were able to clear the lungs of the invading bacteria within 5 days after infection with no involvement of the other organs

ACKNOWLEDGMENT

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INTRODUCTION

It has been shown that acute whole-body X-irradiation lowers the resistance of mice to infection with avirulent Pasteurella tularensis (Live Vaccine Strain LVS) (Hodge and Silverman, 1968). However, the effect of continuous exposure to low dose-rate gamma radiation has not been studied as thoroughly. It is essential to obtain such information in order to make reasonable estimates on the medical and epidemiological problems that might be encountered by both military and civilian populations who must carry out their tasks in fallout areas. There is considerable military interest regarding the possibility that exposure to low dose rate gamma radiation may decrease the individual's resistance to a live avirulent immunizing agent to the extent that serious illness or death might result from the immunization itself. Also, the question has been raised as to whether the ability to acquire immunity might be impaired by exposure to low dose rate gamma radiation such as might be encountered in a radiation fallout field following the use of a nuclear weapon.

Previous studies in this laboratory have shown that mice exposed continuously to Co^{60} gamma irradiation delivered at 1.0 to 1.5 R per hour resulted in a marked increase in susceptibility to infection with airborne Listeria monocytogenes (Stewart, et al 1965) and decreased their immunity at higher radiation doses (Stewart, et al 1966). The importance of these findings in situations where living avirulent strains of organisms are used for immunization has led to an emphasis on this problem using the avirulent Live Vaccine Strain of Pasteurella tularensis as a model. The

proposed use of this organism for immunization of military and civilian populations has posed the question of whether the general increased susceptibility to infection following exposure to either acute whole body or continuous low dose rate radiation renders the administration of the vaccine inadvisable under such conditions. In addition, the question of whether the irradiated individual will develop an immune response to the vaccine must be determined.

MATERIALS AND METHODS

In these experiments, mice were exposed to continuous low dose rate gamma radiation from a Co^{60} source followed by subcutaneous injections with varying doses of the live vaccine strains of Pasteurella tularensis. Immunized survivors were subsequently challenged 30 days later with an airborne infection of either the LVS strain or the virulent SCHU S-5 strain of P. tularensis.

MICE*

Male LAF_1 (C57L ♀ X A/HeJ ♂) mice from our laboratory colony were used in the experiments. The mice were 10 to 12 weeks old and the average weight was 25 grams at the time they were exposed to the Co^{60} source.

IRRADIATION

Mice were exposed to continuous γ radiation from a Co^{60} source at a dose rate of 1.4 R/hour until the desired accumulated doses were obtained. Plastic cages housing ten mice each were placed on curved wooden racks

*In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

so that the center of each was equidistant from the Co^{60} source. Doses were measured with thermoluminescent dosimeters (LiF), Victoreen R-chamber, and Dupont films (No. 555 and 1290). The Co^{60} source was in continuous operation except for 30 minutes per week when the cages were changed. Fresh food pellets and water were also supplied at this time. No deaths occurred among the mice during the radiation exposure period or among animals held as long as sixty days after removal from the Co^{60} source.

IMMUNIZATION

Lyophilized Pasteurella tularensis, Live Vaccine Strain (LVS) was obtained from Fort Detrick, Frederick, Maryland. This strain, although avirulent for guinea pigs, monkeys and man, is virulent for mice when administered as an aerosol. The lyophilized organisms were resuspended in a 0.1% gelatin and 0.85% saline solution and cultured on Difco-Civil Defense Agar enriched with Difco-hemoglobin. After incubation at 37°C for 72-96 hours, the organisms were washed from the agar plages, concentrated by centrifugation at 5000 RPM for 20 minutes and resuspended in gelatin-saline.

The mice were injected subcutaneously with 0.2 ml suspensions of the organisms at concentrations varying from 7.0 to 4×10^9 within one hour after removal from the Co^{60} source. Plate counts were made for each experiment in order to establish the concentration of the organisms injected. Mice were checked daily for illness and deaths. Sick or moribund mice were periodically sacrificed and cultures made of the lung, liver,

spleen, lymph node, and heart blood to determine the presence of Pasteurella tularensis.

CHALLENGE

AVIRULENT AEROSOL

In order to test the immune response, surviving mice were challenged 30 days after immunization with a respiratory infection of Pasteurella tularensis (LVS) given as an aerosol generated by a modified Henderson apparatus described by Pribnow and Silverman (1963). Media and growth conditions used in the cultivation of P. tularensis (LVS) for the aerosol was identical with that used for the immunization procedures with the exception of the addition of 0.1% antifoam (Dow Corning antifoam B) to the final bacterial suspension in the atomizer. The aerosol was sampled with AGI-30 glass impingers and the inhaled dose calculated by use of Guyton's formula (Guyton, 1947). Animals were periodically sacrificed immediately after an aerosol exposure, the lungs removed, homogenized, and plate counts made to check the inhaled dose. In all cases, the number of bacteria in the lung and the calculated inhaled dose were within ± 10% of each other.

VIRULENT AEROSOL

For these experiments, surviving mice were exposed 30 days after immunization to a bacterial aerosol of the virulent SCHU S-5 strain of Pasteurella tularensis at the Naval Biological Laboratory, Oakland, California. The P. tularensis (SCHU S-5) was grown in a blood free (SB) broth (Won, 1953) for 24 hours, centrifiged, and resuspended in fresh

SB broth. An aerosol exposure unit (ABX) was employed to generate the aerosol. The apparatus and procedure was essentially the same as that described by Akers, et al, 1960. Infected animals were observed for 30 days and impression cultures on SB agar were made of heart, lung, liver, and spleen from some of the surviving animals.

BACTERIOLOGICAL STUDIES

Bacterial enumeration of the organism in the lung, liver, spleen, and lymph nodes was determined in animals infected with an aerosol of the avirulent strains. Mice were sacrificed by cervical dislocation, the organs removed, homogenized in sterile mortar and pestle with sterile sand, and gelatin-saline added in amounts equal to the displacement volumes of each organ. The tissue homogenates were diluted with gel-saline and plated on Civil Defense Agar. Colony counts were made after 72 hours incubation (37°C) and the results expressed as number of viable organisms per ml of tissue homogenate.

CALCULATION OF LD_{50}

In all cases, the LD_{50} was calculated either by the method of Litchfield and Wilcoxin (1949) or with the aid of a UNIVAC 1108 computer programmed for Quantal Response.

RESULTS

The Live Vaccine Strain (LVS) of Pasteurella tularensis was found to be totally avirulent when administered to normal mice via the subcutaneous route. No deaths were observed in mice that received doses ranging from 7.0×10^0 to 4×10^6 bacteria/mouse. Only 28% of the mice

injected with 4.0×10^7 organisms died and 33% died after the administration of 4.0×10^9 cells. From this, the LD_{50} for normal mice subcutaneously injected with P. tularensis (LVS), was estimated to be in the range of 10^{15}.

Continuously irradiated mice, however, proved to be much more susceptible to infection after subcutaneous injections of P. tularensis (LVS). As shown in Figure 1, the susceptibility to infection increased as the total amount of radiation increased. For comparison, the LD_{50} of each radiation group is plotted against the total radiation received in Figure 2. Here the LD_{50} decreased as the radiation dose increased.

Eigelsbach, et al (1961) have reported that guinea pigs and monkeys vaccinated with live tularemia vaccine by the respiratory route showed higher levels of antibody and comparable or greater protection against virulent challenge than did animals vaccinated dermally. Attempts to make such comparisons using mice were not possible, since the live vaccine strain was found to be quite virulent in mice when administered as an aerosol. From the data in Table I, the LD_{50} for normal mice exposed to an aerosol of P. tularensis (LVS) was determined to be 1.5×10^3 inhaled bacteria per mouse. Only one experiment involving irradiated mice yielded satisfactory results, that is, enough immunized survivors for later challenge experiments. The inhaled dose was 9.4×10^1 organisms/mouse. The results for these immunization exposures are shown in Table II.

Bacterial enumeration of the lungs, liver, spleen, and lymph nodes of normal mice exposed to a lethal aerosol of P. tularensis (LVS) is

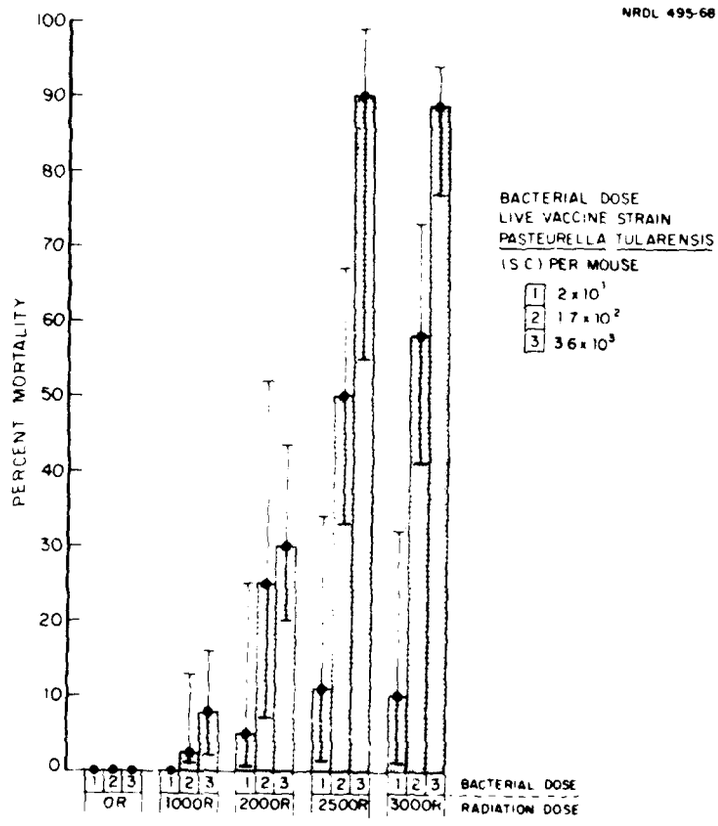

Figure 1. Susceptibility of irradiated mice to infection from subcutaneous injections with the avirulent Live Vaccine Strains of Pasteurella tularensis.

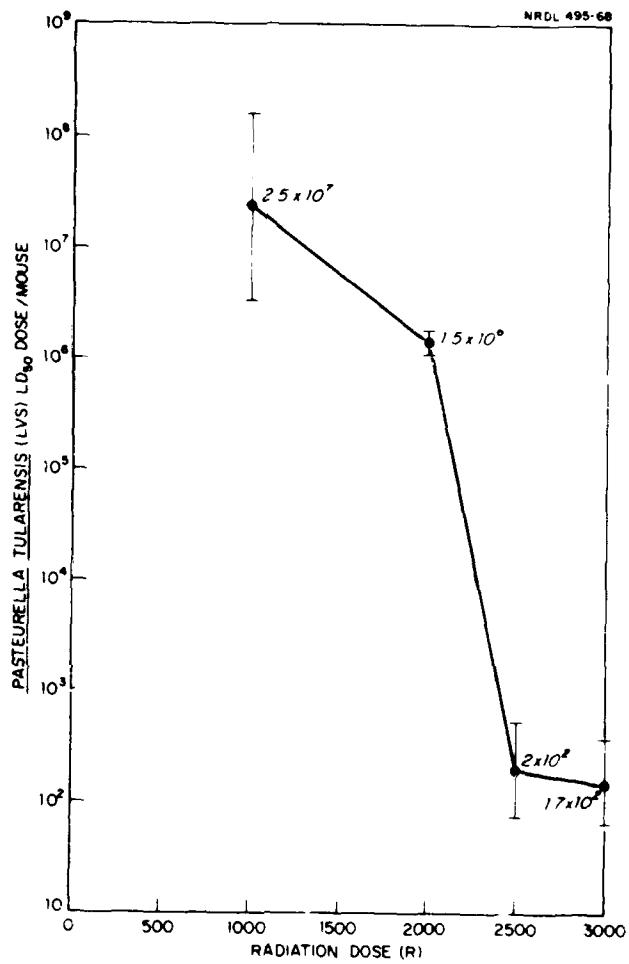

Figure 2. LD_{50} for irradiated mice inoculated (subcutaneous) with the avirulent Live Vaccine Strain of Pasteurella tularensis.

TABLE I

MORTALITY OF NON-IRRADIATED MICE EXPOSED TO AN AEROSOL OF THE AVIRULENT LIVE VACCINE STRAIN OF PASTEURELLA TULARENSIS.

LVS Inhaled Dose	Dead/Total	% Dead	95% Conf. Limits
9.4×10^1	0/20	0	0.00-16.84
2.6×10^2	3/35	8.6	1.80-23.06
8.2×10^2	10/35	28.6	14.64-16.30
3.6×10^3	25/33	76.0	57.74-88.91
3.8×10^3	15/19	79.0	54.43-93.95
1.6×10^4	33/33	100.0	89.42-100.00

TABLE II

MORTALITY OF IRRADIATED MICE EXPOSED TO AN AEROSOL OF THE AVIRULENT LIVE VACCINE STRAIN OF PASTEURELLA TULARENSIS*

Radiation Dose**	# Dead/Total	% Mortality	Conf. Limits
1000 R	3/20	15%	3.21-37.89
1500 R	10/20	50%	27.20-72.80
2000 R	13/20	65%	40.78-84.61
None	0/20	0%	0.00-16.84

*Inhaled dose: 9.4×10^{1} organisms/mouse.
**Radiation dose rate: 1.4 R/hour.

tabulated in Table III. The lungs were the first to become infected followed on the 2nd day post-aerosol by the liver and spleen. The regional lymph nodes did not appear infected until the 4th day after exposure. Irradiated non-immune mice (Table IV) showed essentially the same bacterial growth in all the tissues examined. In all cases, the bacterial number increased until the animal died at an average of 10 days after infection.

Both irradiated immune and normal immune animals (Table IV), however, were able to clear the lungs of bacteria by the 5th day after aerosol exposure. Involvement of the liver, spleen, or lymph nodes was not observed in any of the immune animals examined.

As shown in Table V, the only immune mice that did not survive respiratory challenge with an aerosol of P. tularensis (LVS) were those that received a low subcutaneous immunization dose of the LVS. Those immune and non-immune animals that did succumb began dying on the 8th day and were all dead by the 14th day after the aerosol challenge. No difference was noted in time of death after infection in irradiated animals and non-irradiated mice.

Since irradiated mice immunized with a subcutaneous injection of LVS were found to be immune to subsequent respiratory infection with LVS. Additional groups were challenged with the virulent strain (SCHU S-5) of P. tularensis. The LD_{50} of SCHU S-5 for non-irradiated, non-immune mice was found to be 8.0×10^1 inhaled bacteria/mouse and 3.5×10^3 for non-irradiated immune mice (Figure 3). In challenging the irradiated immune

TABLE III

RECOVERY OF PASTEURELLA TULARENSIS (LVS)[1] FROM NORMAL MICE[4] EXPOSED TO A LETHAL AEROSOL[3]

Day Post Infection	Lung (3 ml)	Liver (6 ml)	Spleen (2 ml)	Axillary Lymph Nodes (1 ml)
1	4×10^4	0	0	0
2	$> 1 \times 10^5$	3.1×10^3	2.3×10^3	0
3	TNC[2]	1.7×10^4	1.0×10^4	0
4	TNC	$> 1 \times 10^5$	$> 1 \times 10^5$	5×10^2
5	TNC	TNC	TNC	$> 1 \times 10^5$
7	TNC	TNC	TNC	TNC

(1) Counts are organisms/ml of homogenized tissue.
(2) TNC = Too numerous to count.
(3) Aerosol dose = 1.7×10^5 inhaled LVS cells.
(4) Five animals autopsied each day.

TABLE IV

RECOVERY OF PASTEURELLA TULARENSIS (LVS) FROM IRRADIATED IMMUNIZED MICE EXPOSED TO AN AEROSOL CHALLENGE

Day Post-Infect→	Irradiated-Immunized 1000 R Imm. Dose = 1.6×10^2 Chal. Dose = 8.3×10^4			Irradiated-Immunized 2000 R Imm. Dose = 1.1×10^2 Chal. Dose = 1.8×10^4				Irradiated-Immunized 3000 R Imm. Dose = 2.4×10^2 Chal. Dose = 7.0×10^5				Irradiated Non-Immunized 2000 R Imm. Dose = None Chal. Dose = 1.8×10^4			
	2	5	7	2	4	6	8	2	4	6	8	2	4	6	8
Lung	$2x10^3$	$2x10^2$	0	$1.9x10^3$	0	0	0	$7.6x10^3$	0	0	0	$1.3x10^3$	$7x10^5$	$5.1x10^4$	$2.7x10^5$
Liver	0	0	0	0	0	0	0	0	0	0	0	0	$6x10^2$	$1.3x10^3$	$7x10^4$
Spleen	0	0	0	0	0	0	0	0	0	0	0	0	$1x10^4$	$2.0x10^4$	$7.8x10^4$
Lymph(A)	0	0	0	0	0	0	0	0	0	0	0	0	$1x10^2$	0	0
Lymph(B)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Day Post-Infect→	Non-Irradiated Immunized Imm. Dose = 1.6×10^2 Chal. Dose = 8.3×10^4			Non-Irradiated Immunized Imm. Dose = 1.1×10^2 Chal. Dose = 1.8×10^4				Non-Irradiated Immunized Imm. Dose = 2.4×10^2 Chal. Dose = 7.0×10^5				Non-Irradiated Non-Immunized Chal. Dose = 8.3×10^4		Non-Irradiated Non-Immunized Chal. Dose = 7×10^5	
	2	5	7	2	4	6	8	2	4	6	8	2	5	2	4
Lung	$1.6x10^3$	0	0	0	0	0	0	$2x10^4$	$3x10^2$	0	0	$7x10^5$	TNC	$9.4x10^6$	$2.9x10^6$
Liver	0	0	0	0	0	0	0	0	0	0	0	$2.6x10^3$	$8.5x10^5$	$1.1x10^4$	$5.7x10^4$
Spleen	0	0	0	0	0	0	0	0	0	0	0	$1.7x10^3$	$2.0x10^4$	$1.8x10^4$	$3.6x10^5$
Lymph(A)	0	0	0	0	0	0	0	0	0	0	0	$3x10^2$	0	$1.6x10^3$	0
Lymph(B)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Lymph(A) = Mediastinal Node
Lymph(B) = Axillary Node
NOTE: 5 Animals autopsied each day

TABLE V

MORTALITY OF IRRADIATED, IMMUNIZED MICE EXPOSED TO AN AEROSOL CHALLENGE WITH THE AVIRULENT LIVE VACCINE STRAIN OF PASTEURELLA TULARENSIS

Immunizing Dose	Challenge Dose	NRNI		NRI		1000 R		2000 R		2500 R		3000 R	
		D/T	%M	D/T	%M	D/T	%M	D/T	%M	D/T	%M	D/T	%M
1.6×10^1	7.8×10^4	9/9	100	6/10	60	7/17	41						
1.6×10^1	3.1×10^4	10/10	100	0/10	0					5/16	24		
2.4×10^1	7.0×10^5	10/10	100	2/10	20							3/18	17
1.1×10^2	2.1×10^4	10/10	100	0/10	0			0/16	0				
1.6×10^2	8.3×10^4	9/9	100	0/8	0	2/17	11						
1.6×10^2	4.1×10^5	10/10	100	0/10	0					0/19	0		
2.4×10^2	7.0×10^5	9/9	100	0/10	0							0/16	0
1.1×10^3	2.0×10^4	10/10	100	0/10	0			0/25	0				
1.6×10^3	1.0×10^5	10/10	100	0/10	0								
1.6×10^3	1.8×10^4	8/11	73	0/10	0					0/8	0		
1.1×10^4	8.6×10^3	8/10	80	0/10	0			0/18	0				

NRNI = Non-Irradiated Non-Immune
NRI = Non-Irradiated Immunized
D/T = Number Dead/Total
%M = % Mortality

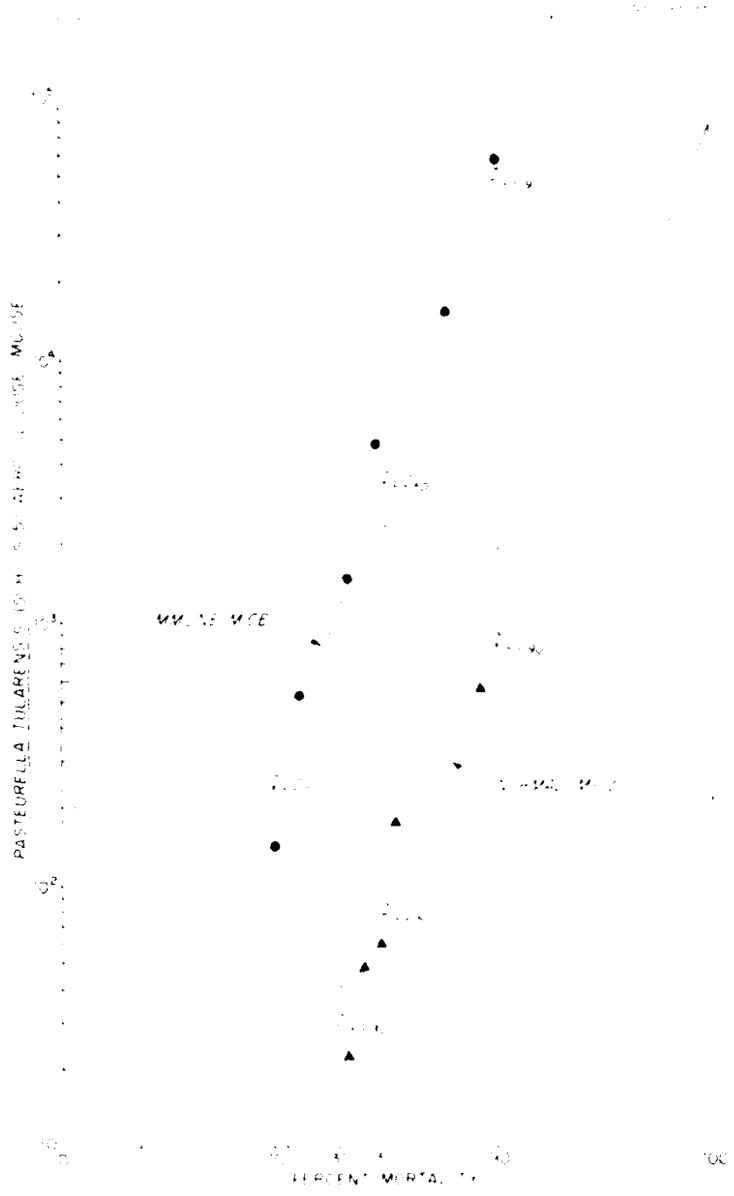

Figure 3. LD_{50} for normal and immune mice exposed to an aerosol challenge with the virulent SCHU S-5 strain of Pasteurella tularensis.

mice with the virulent SCHU S-5, it was not possible to use the same culture of SCHU S-5 for all experiments, consequently there is a considerable variation in the results. However, a representative experiment is shown in Table VI. In this experiment, the immunity is decreased as the total amount of radiation is increased. Other experiments showed less depression of the immune response and some showed more. However, when the data from all the experiments were compiled, irregardless of immunizing dose or challenge dose, the trend toward an impaired immunity at the higher radiation doses becomes obvious (Table VI). This compiled data is shown graphically in Figure 4. The immunizing doses ranged from 2×10^2 to 3.6×10^3 LVS cells injected per mouse and the challenge doses varied from a low of 8.5×10^1 to a high of 9×10^3 SCHU S-5 cells inhaled per mouse.

Normal mice given an immunizing dose of 3.6×10^3 LVS subcutaneously and challenged 30 days later with the virulent SCHU S-5 strain were sacrificed 30 days after challenge. Although these animals showed no obvious indications of infection, SCHU S-5 was isolated from the heart blood, lungs, liver, and spleen (Table VII). Positive identification of the organism was confirmed by agglutination and virulence in normal mice as well as the normal bacteriological procedures for identification.

DISCUSSION

The data presented, revealed that Pasteurella tularensis (LVS) injected subcutaneously into normal non-irradiated mice did not cause an

TABLE VI

MORTALITY OF IRRADIATED, IMMUNIZED MICE EXPOSED TO AN AEROSOL CHALLENGE WITH THE VIRULENT SCHU S-5 STRAIN OF PASTEURELLA TULARENSIS

Immunizing Dose	Challenge Dose	NRNI**		NRI***		1000 R		1500 R		2000 R		2500 R		3000 R	
		D/T	% M	D/T	% M	D/T	% M	D/T	% M	D/T	% M	D/T	% M	D/T	% M
1.5×10^3	1.8×10^2	20/20	100	1/17	6	2/20	10			11/24	46			15/18	84
2.6×10^2	7.1×10^2	28/28	100	5/28	18	17/43	40			19/28	68			21/21	100
*$2x10^2$-$3.6x10^3$	$8.5x10^1$-$9x10^3$	272/278	98	147/275	53	62/110	56	30/52	58	51/105	49	36/39	92	50/60	84

*Compiled results from all experiments.
**Non-Irradiated, Non-Immune.
***Non-Irradiated, Immune.

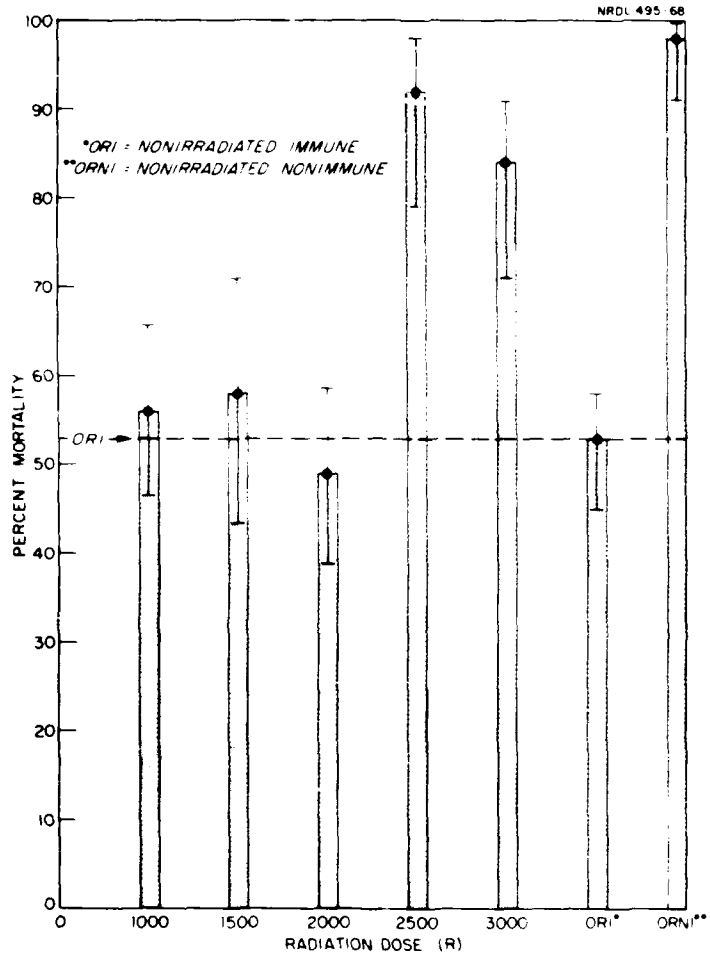

Figure 4. Mortality of irradiated, immunized mice exposed to an aerosol challenge with the virulent SCHU S-5 strain of Pasteurella tularensis.

TABLE VII

RECOVERY OF PASTEURELLA TULARENSIS (SCHU S-5) FROM IRRADIATED AND NON-IRRADIATED IMMUNE MICE EXPOSED TO AN AEROSOL CHALLENGE OF SCHU S-5

Immunizing Dose**	Challenge Dose***	Total Amount of Radiation Rec'd (in R)****	Total Number of Animals Sacrificed	Number of Animals with Positive Cultures	
				40 Days Post-Challenge	50 Days Post-Challenge
4.8×10^2	2×10^2	0	8	2	0
4.8×10^2	2×10^2	1000	8	2	2
4.8×10^2	2×10^2	0	10	3	2
4.8×10^2	2×10^2	2000	8	2	0
4.8×10^2	2×10^2	0	6	3	3
4.8×10^2	2×10^2	3000	7	0	1
4.8×10^2	2×10^3	0	1	1	-
4.8×10^2	2×10^3	2000	1	0	-
4.8×10^2	2×10^3	0	2	1	-
1.6×10^3	3×10^2	0	15	4	-
1.6×10^3	1.9×10^3	0	9	6	-

*Impression smears of the tissue on agar plates.
**Subcutaneous injection with the Live Vaccine Strains of P. tularensis.
***Inhaled SCHU S-5 strain of P. tularensis.
****Radiation Dose Rate: 1.4 R/hour.

active infection but acted as an immunizing vaccine as was shown by Eigelsbach and Downs (1961). Animals exposed to low dose rate γ radiation, however, proved to be much more susceptible to the same organism. Although animals that received 1000 R did not show an appreciable increase in susceptibility to infection, any increase becomes significant ($P = 0.05$) since non-irradiated mice injected with a similar number of organisms survived the injections (Fig. 1). The increase in per cent mortality from tularemia was greater at 2000 R, and at 2500 R and 3000 R obviously increased. There was no significant difference between 2500 R and 3000 R. Hammond, et al (1959) found that mice exposed to continuous γ irradiation from Co^{60} at dose rates ranging from 34 to 130 R per day were more susceptible to infection with Pseudomonas aeruginosa, but that the increase in susceptibility was dependent upon dose rate rather than the total amount accumulated. The dose rate in our experiments was kept constant at 33.6 R per day. Therefore, the effects of radiation must be assumed to be due to the total dose received since the susceptibility to infection with tularemia increased as the total radiation dose increased. This premise was also found to be true for acutely x-irradiated mice infected with P. tularensis vaccine (Hodge and Silverman, 1968).

Chamberlain (1965) found that one of the characteristics of live tularemia vaccine was that it shows some virulence for mice. This fact may explain why the LVS was so virulent for mice when administered as an aerosol. Both irradiated and non-irradiated immune mice, however, were able to survive high aerosol doses of inhaled LVS cells. This

demonstrates a good specific immune response. The only immunized animals, both irradiated and normals that did not show 100% survival after respiratory challenge were those that received a low immunization dose ($< 2 \times 10^2$). The aerosol experiments with the vaccine strain provided an excellent means for studying the bacteriology and pathology of the tularemia infection. The findings from the bacterial enumerations were similar to those found by other workers in the guinea pig by Nutter and Eigelsbach (1967), and in the monkey by Eigelsbach, et al (1962) and by White, et al (1962).

In the irradiated immune mice challenged with the virulent SCHU S-5 strain, there was an increase in mortality as the total radiation dose increased. As shown in Figure 4, immune animals that received radiation doses of 1000 - 2000 R survived aerosol challenge as well as non-irradiated animals. However, above 2000 R, the survival was not significantly different from non-irradiated, non-immune controls. Stoner and Hale (1963) state that active and passive immunity to pneumococcal infections were severely depressed in pathogen-free Swiss albino mice exposed to continuous low level γ radiation at 1 or 4 R/hour. They concluded that the repression of active and passive immunity resulted from damage to the cellular defense mechanism of the host. Our findings are in concert with this hypothesis. Experiments involving acute sublethal x-irradiation of mice and guinea pigs immunized with a vaccine strain of F. tularensis showed only a moderate reduction in intensity of immunity (Shevelev and Prudnikova, 1963), (Nutter and Eigelsbach, 1967), and (Nutter and Guss, 1967).

It is interesting to note that it was possible to culture the virulent SCHU S-5 organism from immune mice that had apparently recovered from the virulent challenge. A speculative explanation for this is that there is a "toxic factor" present in the virulent challenge organism against which the vaccine strain protects, leaving the virulent organism itself a relatively inocuous parasite in the host. Öz(1940) suggested the presence of an endotoxin in tularemia organisms and Nicholes and Manilla (1954) reported a "toxin" or toxic substance isolated from the cell soma of the organism.

It is our conclusion that although continuous low dose rate γ-radiation does not produce overt illness even at high total doses, any further insult, such as a live bacterial vaccine will result in serious illness. Should animals survive the immunization after irradiation, some impairment of immunity may occur.

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13. ABSTRACT

Mice were exposed to continuous low dose rate (1.4 R/hr) γ radiation. Immediately after accumulating 1000 R, 2000 R, or 3000 R, they received a subcutaneous injection of the live vaccine strain (LVS) of Pasteurella tularensis. Although the avirulent strain caused no effect in normal mice, a fulminating infection occurred in irradiated mice. As the total radiation dose increased the LD_{50} of the organism decreased.

Animals surviving irradiation and immunization were subjected to an aerosol challenge of either the avirulent LVS or the virulent SCHU S-5 strain of P. tularensis. The LVS strain is virulent for mice when administered as an aerosol, but not when injected subcutaneously. Although an aerosol of avirulent organisms was lethal for normal mice, immune mice, both non-irradiated and irradiated, survived an aerosol infection with the avirulent organism. Irradiated, immunized mice challenged with an aerosol of the virulent SCHU S-5 strain showed a decrease in immunity, especially at the higher levels of radiation.

Bacterial counts of the lungs, liver, spleen, and lymph nodes of non-immunized mice exposed to a lethal aerosol dose of the avirulent P. tularensis (LVS) showed increasing numbers of bacteria after the 2nd post-infection day. This increase continued until the death of the animal on abouth the 10th day. Immune animals, however, were able to clear the lungs 5 days after infection with no involvement of the other tissues.

Although continuous low dose rate γ radiation does not produce the gross radiation pathology that one sees following acute radiation, exposre to a live avirulent Pasteurella tularensis vaccine produces an infection and an imparied immunity.

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